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Short communication: Relationship between the efficiency of utilization of feed nitrogen and ^{15}N enrichment in casein from lactating dairy cows

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ABSTRACT

The objective of the present study was to evaluate the relationship between the efficiency of conversion of feed N into milk N [N-use efficiency (NUE)] and ^{15}N enrichment of milk casein from lactating cows fed corn silage-based diets. Samples of feeds and milk were obtained from 3 experiments with lactating dairy cows. All diets were based on corn silage and were designed to evaluate the effects of (1) diets with different ratios of effective rumen-degradable protein to fermentable metabolizable energy (experiment 1), (2) different proportions of quickly and slowly rumen-degradable protein (experiment 2), and (3) synchronizing the availability of fermentable metabolizable energy and effective rumen-degradable protein in the rumen (experiment 3). Although no significant effect of diet on casein δ^{15}N values was detected, casein was more enriched than the diet in each of the experiments. Nitrogen-use efficiency was negatively related to adjusted Δ^{15}N (casein δ^{15}N − diet δ^{15}N) for experiments 1 and 2 individually and when combining data from all 3 experiments. The relatively low values for Δ^{15}N suggest that these productive animals were using dietary N efficiently, with a high proportion of N going to milk protein and less to urea. The weak, although significant, relationship between NUE and adjusted Δ^{15}N, is consistent with relatively little variation in hepatic deamination and transamination, with variation in rumen efficiency having the predominant effect on NUE. The present study confirms the lower ^{15}N enrichment in protein when NUE is high and the potential to use N-isotope fractionation as a marker of NUE.

Key words: dairy cow, feed efficiency, nitrogen-15, isotopic fractionation

Short Communication

Conversion of plant protein into protein within milk and meat is the basis of profitable and environmentally sustainable ruminant production systems. Many papers have reported diet effects on the efficiency of conversion of feed protein into milk protein [N-use efficiency (**NUE**)]. Nitrogen-use efficiency has been evaluated through feeding studies and the N balance technique (see review by Castillo et al., 2000). Recently, new focus has been on animal genetic and genomic effects on feed conversion efficiency, of which NUE is an important part (Moore et al., 2009). These studies require estimates of feed conversion efficiency or NUE from large numbers of animals, so we are exploring alternative marker-based approaches to characterize variation in feed efficiency. These methods need to be low cost and use accessible samples.

One such approach is based on the phenomenon of N isotopic fractionation. The mass differences between molecules containing ^{14}N and ^{15}N results in an isotopic fractionation in various biochemical pathways, with milk becoming enriched in ^{15}N relative to the diet and urine depleted (Deniro and Epstein, 1981; Sutoh et al., 1987). Isotopic fractionation occurs during transamination and deamination reactions that result in the conversion of excess protein into urea in the liver (Balter et al., 2006), protein turnover and recycling (Martínez del Rio and Carleton, 2012), and rumen metabolism (Wattiaux and Reed, 1995). Fractionation within the liver results in an inverse relationship between diet protein quality and increased ^{15}N in animal proteins (Sick et al., 1997). Fuller et al. (2004) showed a similar negative relationship between ^{15}N in hair and weight gain in pregnant women. Sponheimer et al. (2003) provided preliminary evidence for the same relationship, with increased ^{15}N enrichment of hair protein for ruminant animals fed high-protein diets (19%) than low-protein diets (9%). The degree of fractionation depends on the efficiency of use of dietary N; that is, on the efficiency of assimilation (Martínez del Rio and Carleton, 2012) and metabolic rates (Smith et al., 2010). Indeed, some studies with mature ruminants (Sutoh et al., 1993;

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Sponheimer et al., 2003), which use dietary N less efficiently, reported a high degree of isotopic fractionation, whereas studies with young or lactating animals (Sutoh et al., 1993; Jenkins et al., 2001) reported lower isotopic fractionation. Regarding lactating dairy cows, the relationship between NUE and N isotopic fractionation was not confirmed in a previous study with milk protein from cows (Cheng et al., 2011). It seems likely that the lack of relationship was the result of N isotopic fractionation when ammonia-N (rather than amino acid N) was incorporated into microbial N (Wattiaux and Reed, 1995) because some of the forages used by Cheng et al. (2011) contained a high proportion of ammonia-N. The objective of the present study was further evaluation of the relationship between NUE and the ^{15}N enrichment of milk protein from lactating cows fed corn silage-based diets.

Samples of feeds and milk were obtained from 3 experiments with lactating dairy cows, designed according to three 3 × 3 Latin squares, and reported by Cabrita et al. (2003). All diets were based on corn silage and were designed to (1) study the effects of diets with different ratios of effective RDP (**ERDP**) to fermentable ME (**FME**; experiment 1), (2) evaluate effects of different proportions of quickly and slowly RDP (experiment 2), and (3) investigate effects of synchronizing the availability of FME and ERDP in the rumen (experiment 3). Briefly, in experiment 1, 6 multiparous and 3 primiparous Holstein cows, averaging 149 DIM (SD = 13.0) and producing 29 kg of milk/d (SD = 4.6), were fed TMR containing (DM basis) approximately 40% corn silage, 5% coarsely chopped ryegrass hay, and 55% concentrate. Diets were formulated to be isoenergetic and to satisfy MP requirements, and they differed in the ERDP:FME ratio (6.7, 10.1, and 11.2 g of ERDP/MJ of FME for treatments with a large deficiency, a slight deficiency, and a slight excess, respectively). Experiment 2 used 6 multiparous and 3 primiparous Holstein cows, averaging 63 DIM (SD = 31.3) and producing 34 kg of milk/d (SD = 5.0). Three isoenergetic and iso-ERDP TMR diets (35% corn silage, 5% coarsely chopped ryegrass hay, and 60% concentrate, on a DM basis) were formulated to differ in the quickly RDP:slowly RDP ratio, achieved by replacing soybean meal with urea in the concentrates [0, 0.5, and 1.0% urea content (**UC**)]. In experiment 3, 3 multiparous and 6 primiparous Holstein cows, averaging 127 DIM (SD = 31.0) and producing 29 kg of milk/d (SD = 5.9), were used. To avoid confounding between rumen synchrony of N and energy availabilities and diet ingredients, all treatments were based on (DM basis) corn silage (45%), coarsely chopped ryegrass hay (5%), energy-rich concentrate (35%), and protein-rich concentrate (15%). Forages and energy-rich concentrate were fed as a partial mixed ration; the protein-rich concentrate was fed either once (before the morning meal) or twice (before both meals) per day, or as a TMR given with the basal diet.

Milk was sampled at both milkings on 2 consecutive days during the last week of each experimental period (Cabrita et al., 2003), and casein was immediately extracted according to the method described by Wilson et al. (1988) and dried in an oven at 90°C for 24 h. The ^{15}N:^{14}N ratios of feeds and casein were determined by isotope ratio mass spectrometry (PDZ Europa Ltd., Rudheath, Northwich, UK). The isotopic composition of samples was expressed in delta units (δ^{15}N; ‰), relative to atmospheric ^{15}N/^{14}N (0.3663%; Mariotti et al., 1981).

Dietary treatment effects on the δ^{15}N of the diet and casein from the 3 experiments were analyzed as three 3 × 3 Latin squares using PROC MIXED of SAS (version 9.1; SAS Institute Inc., Cary, NC). The model included the fixed effects of square, period, and diet, the random effect of cow within square, and the random residual error. Difference in isotopic fractionation (termed Δ^{15}N; δ^{15}casein − δ^{15}diet) were related to NUE using a mixed-model regression analysis, according to St-Pierre (2001). Covariance structures for the intercepts and slopes were chosen according to the finite sample corrected Akaike information criterion and the Schwarz Bayesian information criterion (Wang and Goonewardene, 2004). Adjusted observations were calculated by adding the residual from each individual observation to the predicted value of the study regression (St-Pierre, 2001). These adjusted observations were corrected for cow within square or cow within experiment for individual analysis and when considering data from all experiments, respectively.

Regarding the experimental feeds, the corn silage used in experiment 1 had a lower δ^{15}N value than those used in experiments 2 and 3 (Table 1). The δ^{15}N values of concentrates used in experiment 1 were higher than those of that used in experiment 2, with the slight-excess and 1.0% UC diets having the lowest values within each experiment. In experiment 3, the energy-rich concentrate had a higher δ^{15}N value than the protein-rich concentrate.

Treatment effects on dietary CP level, NUE, and δ^{15}N of the whole diet, casein, and Δ^{15}N (the difference between δ^{15}N of casein and the diet) are presented for each of the 3 experiments in Table 2. The dietary CP level in experiment 1 increased with the increase in ERDP:FME ratio and, in experiment 2, the 1.0% UC diet had the highest CP level. The NUE was significantly higher for the large-deficiency diet (experiment 1), and lowest for the 1.0% UC diet (experiment 2), agreeing with the well-known direct relationship between N excretion in milk and intake of protein (Cabrita et al., 2007, 2011; Nadeau et al., 2007). Additionally, when the dietary N

Table 1. δ^{15} Nitrogen values (SD in parentheses) of feeds used for the 3 experiments

	Experiment 1[1]					Experiments 2 and 3		Experiment 2[2]			Experiment 3[3]	
Item	CS	H	RL	RM	RH	CS	H	U0	U5	U10	ER	PR
δ^{15}N	1.64 (0.397)	0.56 (0.556)	3.01 (0.097)	3.20 (0.167)	2.63 (0.195)	3.78 (0.343)	1.25 (1.068)	2.14 (0.136)	2.30 (0.127)	1.93 (0.266)	2.91 (0.412)	1.60 (0.434)

[1]CS = corn silage; H = ryegrass hay; RL, RM, and RH = concentrates with a large deficiency, slight deficiency, and slight excess, respectively, according to the effective RDP:fermentable ME ratio of the diet.

[2]Concentrates were named according to their urea content: U0 = 0%, U5 = 0.5%, and U10 = 1.0%.

[3]Concentrates were named according to the main nutrients supplied: ER = energy-rich nutrients and PR = N-rich nutrients.

Table 2. Least squares means of dietary CP concentration (after correction for orts), efficiency of conversion of feed N into milk N [N-use efficiency (NUE)], δ^{15}N of diet and casein, and Δ^{15}N (casein δ^{15}N − diet δ^{15}N) from the 3 experiments

	Experiment 1[1]					Experiment 2[2]					Experiment 3[3]				
Item	RL	RM	RH	SEM	*P*-value	U0	U5	U10	SEM	*P*-value	DU	D2	D1	SEM	*P*-value
Dietary CP (%)	13.76	15.29	16.93	0.0865	<0.001	16.76	16.73	17.51	0.0720	<0.001	14.44	14.37	14.44	0.0785	0.52
NUE	0.340	0.295	0.268	0.0090	<0.001	0.302	0.298	0.279	0.0105	0.008	0.357	0.341	0.327	0.0121	0.072
δ^{15}N															
Diet	2.64	2.82	2.40	0.027	<0.001	2.38	2.51	2.19	0.043	<0.001	2.76	2.75	2.74	0.006	0.14
Casein	4.67	4.79	4.87	0.180	0.49	4.84	4.84	4.79	0.067	0.82	3.79	3.62	3.75	0.211	0.70
Δ^{15}N															
Casein δ^{15}N − diet δ^{15}N	2.03	1.97	2.46	0.195	0.022	2.45	2.32	2.60	0.091	0.071	1.03	0.86	1.00	0.214	0.70

[1]In experiment 1, diets were named according to the effective RDP:fermentable ME ratio of the diet: RL, RM, and RH provided a large deficiency, slight deficiency, and slight excess, respectively.

[2]In experiment 2, diets were named according to the urea content of the concentrates: U0 = 0%, U5 = 0.5%, and U10 = 1.0%.

[3]In experiment 3, treatments were named according to the mode of distribution of the protein-rich concentrate: DU = as a TMR with the basal diet; D2 = in two meals, immediately before the distribution of the basal diet; and D1 = in one meal, immediately before the distribution of the morning meal.

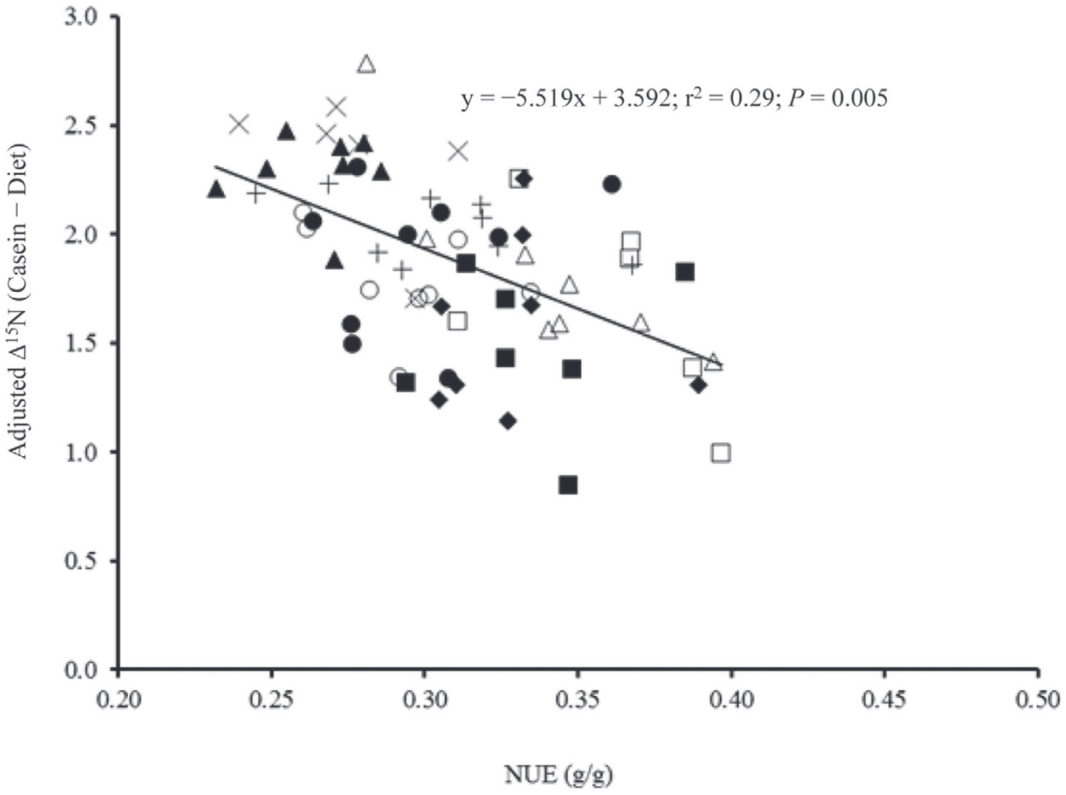

Figure 1. Relationship between the efficiency of conversion of feed N into milk N [N-use efficiency (NUE)] and adjusted Δ^{15}N (casein δ^{15}N − diet δ^{15}N) for experiments 1, 2, and 3. Each data point represents the value for each cow, in each dietary treatment. In experiment 1, diets are named according to the effective RDP:fermentable ME ratio of the diet: RL (Δ), RM (○), and RH (▲) provided a large deficiency, a slight deficiency, and a slight excess, respectively. In experiment 2, diets are named according to the urea content of the concentrates: U0 = 0% (+), U5 = 0.5% (●), and U10 = 1.0% (×). In experiment 3, treatments are named according to the mode of distribution of the protein-rich concentrate: DU = as a TMR with the basal diet (□); D2 = in 2 meals, immediately before the distribution of the basal diet (■); and D1 = in 1 meal, immediately before the distribution of the morning meal (◆).

supply is low, the increase in reabsorption of urea in the kidneys (Marini and Van Amburgh, 2001) increases the recycling of urea to the rumen, thus increasing NUE. Dietary protein must simultaneously cover the need for N and amino acids of rumen microbes and the amino acid requirements of the cow, the synchronization of the supply of N and C (energy) being important to obtain optimal rumen fermentation and minimal N excretion from ruminants (Cabrita et al., 2006). In experiment 3, NUE tended ($P = 0.072$) to be higher with more synchronized diets (protein-rich concentrate fed as a TMR given with the basal diet and protein-rich concentrate fed twice per day).

The δ^{15}N was highest for the slight deficiency and 0.5% UC diets for experiments 1 and 2, respectively, reflecting differences in the δ^{15}N content of concentrates. As expected, δ^{15}N values were not affected by treatments in experiment 3. In the present study, δ^{15}N values were similar to those for diets based on red clover silage, but lower than those for grass silage-based diets or mixtures (40:60 on a DM basis) of red clover and corn silages in the work reported by Cheng et al. (2011).

Although no significant effect of diet on casein δ^{15}N values was detected, casein was always more enriched than the corresponding diet, agreeing with previous results (Sutoh et al., 1987; Cheng et al., 2011). The difference between casein and diet δ^{15}N values (Δ^{15}N) was higher for diets with higher CP levels, which also resulted in lower NUE (slight excess and 1.0% UC diets). Indeed, dietary CP level significantly correlated with adjusted Δ^{15}N when using data from all experiments ($R^2 = 0.30$; $P = 0.006$), and NUE was negatively related to adjusted Δ^{15}N for experiments 1 ($R^2 = 0.49$; $P = 0.010$) and 2 ($R^2 = 0.067$; $P = 0.027$), individually and when pulling together data from the 3 experiments ($R^2 = 0.29$; $P = 0.005$; Figure 1). No significant relationship existed when data from experiment 3 was analyzed separately, suggesting that the different feeding patterns may have affected the relative contributions of N from the energy- and protein-rich concentrates to milk protein synthesis, thus complicating the relationship with NUE. However, the differences between casein and diet δ^{15}N values observed were lower than those reported for growing or mature animals, and even with lactating

dairy cows in the study by Cheng et al. (2013). It would be expected that more productive animals use dietary N more efficiently, with a higher proportion of N going to milk protein and less to urea. Despite being significantly correlated, the relationships between NUE and adjusted $\Delta^{15}N$ were weaker than the relationship between NUE and nonadjusted $\Delta^{15}N$ in a study with dairy cows reported by Cheng et al. (2013). This is consistent with relatively little variation in hepatic deamination and transamination, with variation in rumen efficiency having the predominant effect on NUE. Indeed, in all 3 experiments, dietary treatments were designed to affect rumen efficiency without a large excess of RDP. However, it must be noted that some diets used in the current study had a lower protein content and urea recycling could have been important. The effect of urea recycling on isotopic fractionation remains unclear. The ammonia resulting from urea recycling can be utilized in the synthesis of NEAA. However, as many α-keto acids can originate from the metabolism of carbohydrates, the recycling of urea can lead to routing of carbon from carbohydrate into protein (Gannes et al., 1998). Additionally, animals on low-protein diets deaminate tissue protein to use as energy and amino acid sources. As ^{14}N is preferentially excreted, the N in animal protein would become ^{15}N-enriched (Hobson et al., 1993).

In conclusion, the present study confirms the lower ^{15}N enrichment in protein when NUE is high and the potential to use N isotope fractionation as a marker for NUE.

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